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Review

Nitric Oxide Synthase Inhibitors as Antidepressants

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Abstract: Affective and anxiety disorders are widely distributed disorders with severe social and economic effects. Evidence is emphatic that effective treatment helps to restore function and quality of life. Due to the action of most modern antidepressant drugs, serotonergic mechanisms have traditionally been suggested to play major roles in the pathophysiology of mood and stress-related disorders. However, a few clinical and several pre-clinical studies, strongly suggest involvement of the nitric oxide (NO) signaling pathway in these disorders. Moreover, several of the conventional neurotransmitters, including serotonin, glutamate and GABA, are intimately regulated by NO, and distinct classes of antidepressants have been found to modulate the hippocampal NO level *in vivo*. The NO system is therefore a potential target for antidepressant and anxiolytic drug action in acute therapy as well as in prophylaxis. This paper reviews the effect of drugs modulating NO synthesis in anxiety and depression.

Keywords: nitric oxide; antidepressants; psychiatry; depression; anxiety

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1. Introduction

Recent data from Denmark and Europe [1,2], indicate that brain disorders account for 12% of all direct costs in the Danish health system and 9% of the total drug consumption was used for treatment of brain diseases. Expenses for brain diseases constituted 3% of the gross national product, and the total expenses for all investigated brain diseases were 37.3 billion DKK. Among brain disorders,

affective disorders were among the most costly diseases, and anxiety disorders among the most prevalent.

The pathogenesis of mood disorders remains elusive, but it is evident that multiple factors, genetic and environmental, play a crucial role for adult psychopathology and neurobiology [3]. With regard to therapy, a significant proportion of affective disorder patients are partial or non responders, and there has been no major breakthrough in finding novel effective drug targets since the introduction of the current marketed antidepressant drugs in the 1950s to the 1980s, which all are based on monoaminergic pharmacological effects. Consequently, there exists a pressing need to develop novel treatment strategies and ultimately understand the etiology and pathophysiology of affective disorders.

Nitric Oxide, originally termed Endothelial-Derived Relaxing Factor (EDRF) before it was discovered that NO and EDRF were the same substance, serves important roles in the cardiovascular system and macrophages [4,5]. In addition, NO has been shown also to have an important role in the nervous system [6,7], where NO serves as a messenger molecule in a number of physiological processes, including processes being linked to the major psychiatric diseases [8–11]. The present paper will review general aspects of the NO system, as well as focus on inhibitors of NO production as putative therapeutic agents towards anxiety and affective disorders.

2. General aspects of Nitric Oxide

NO is a small molecule (MW 30 Da), which *in vitro* is a colorless gas and a product from the breakdown of N_2. NO is degraded into nitrites and nitrates, and depending on the environmental conditions, the half life ranges from minutes to years [12]. The combination of one atom of N and one atom of O, results in the presence of an unpaired electron. However, NO is less reactive than many other free radicals, and does not react with itself. Nevertheless, the compound is known to be an important mediator of cytotoxicity in the immune system [13].

In biological systems the half-life of NO is estimated to be about 30 s or less [12]. The molecule is uncharged and is therefore freely diffusible across cell membranes and other structures. NO is produced and released by many different cells in multicellular organisms and can thus act as a tool for intercellular communication [14,15,16,17,18,19].

2.1. The Nitric Oxide Synthase enzymes

The enzyme responsible for the synthesis of NO, nitric oxide synthase (NOS), appears, in different isoforms which are constitutive or inducible. The activity of the constitutive NOS depends on Ca^{2+} and calmodulin, whereas the inducible NOS are independent from both Ca^{2+} and calmodulin. A distinction of the isoforms is also made based on the tissue where the NOS was identified the first time and primarily located. Of the constitutive isoforms, NOS in endothelial cells is mainly located in the cell membrane, and is termed eNOS. NOS in neuronal cells is located throughout the cell and termed nNOS. The inducible isoform, NOS in the immune system is located in macrophages is termed iNOS and consists of soluble and membrane bound NOS [19,20]. However, exceptions from this rule exist. nNOS has been found in a variety of non-neuronal cells and eNOS have been demonstrated in some neurons [21,22]. The present NOS classification thus consists of three classes, which does not specify

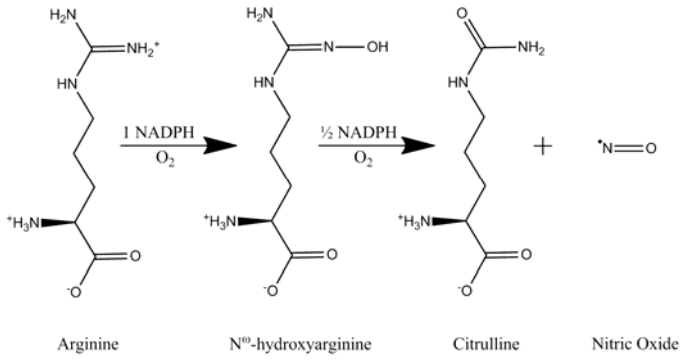
the cells in which they may occur or whether they are induced, but refers to the tissue where the NOS was identified the first time:

- nNOS is the NOS first identified in neurons and which is dependent of elevated Ca^{2+}.
- iNOS is the NOS which is independent of elevated Ca^{2+}.
- eNOS is the NOS first identified in endothelial cells and which is dependent of elevated Ca^{2+}.

2.2. Synthesis of NO

NO is synthesized in the brain by NOS from the amino-acid L-arginine. In brief, L-arginine is converted to N^{ω}-hydroxy-L-arginine, which is further converted to NO and citrulline by NOS (Scheme 1). The process is rather complex and further discussion lies beyond the scope of this text. Briefly, the process involves five electrons, three co-substrates and five prostethic groups [19,23,24].

Scheme 1. Synthesis of Nitric Oxide.



2.3. Localization of NOS in the CNS

The NOS enzymes are widely distributed within the mammalian brain [25,26]. The neuronal isoform accounts for the majority of the NOS activity in the brain [27], and NOS positive neurons are located in the hippocampal layers CA1-CA3, the medial amygdaloid nucleus, the olfactory bulb, the layers II-VI in the cerebral cortex, the granular and deep molecular layers of the cerebellum and, with special interest regarding the serotonin system, in the dorsal and medial raphe nuclei [25]. Measurements of NOS activity in different brain regions have shown the highest activity in the cerebellum, the midbrain, the hypothalamus, the cortex, the striatum and the hippocampus [28,29]. Interestingly, NO has been shown to co-localize with several other known transmitters within the same neuron, e.g. serotonin (5-HT) in the medial and dorsal raphe nuclei [30], Norepinephrine (NE) in the solitarian tract nucleus [31], γ-aminobutyric acid (GABA) in the cerebral cortex [32] and Neuropeptide Y (NPY) and somatostatin in the striatum [33].

It is important to emphasize that certain neurons also contain the eNOS besides the nNOS [34]. The consequences of this finding remain to be determined, but it is likely that neuronal eNOS and nNOS serve different roles in the CNS [35]. Under normal physiological conditions, iNOS in the brain should have no role, in that the activity of iNOS is largely undetectable. However, under pathological conditions, such as trauma, ischemia or infection, iNOS may become important [36].

2.4. Regulation of NOS activity

Regulation of the NOS enzyme expression has to be clarified in detail. Most of the studies performed have focused on the iNOS isoform. This isoform is not present in the cells under normal circumstances, but can be expressed following activation by different cytokines/endotoxines [37,38]. Less is known about the expression of nNOS and eNOS, but it has become evident that expression of nNOS in the brain and spinal-cord during the embryonic and post-natal period can change markedly, which is in line with evidence indicating that NO is implicated in synaptic plasticity in the adult and in regulating neurite outgrowth, as exemplified by the finding that NO donors enhance neurotrophin-induced neurite outgrowth through a cGMP-dependent mechanism and [39–41].

The co factors and especially the NOS-Ca^{2+}-calmodulin interaction is a primary regulator for NO production. Following an action potential, increases in the intracellular Ca^{2+} environment (around 500 nM [42]), triggers Ca^{2+}-calmodulin to bind to NOS, activating the NOS enzymatic activity. As, the intracellular Ca^{2+} level can rapidly change, the catalytic activity can be turned on and off within a short time. These regulatory properties form basis of the understanding of NO as a neurotransmitter. Interestingly, iNOS binds calmodulin very tightly, and continue to synthesize NO thoughout the life of the enzyme, irrespectively of the intracellular Ca^{2+} concentration [19]. In addition to the co-factor and Ca^{2+} level regulations, phosphorylation is used to regulate the activity, as exemplified by the finding that nNOS phosphorylation by protein kinase C inhibits NO production [43]. Finally, NO itself has been shown to regulate NOS activity [44–46]. The nature of this inhibition needs to be fully clarified, but can be hypothesized to involve nitrosylation [47].

2.5. Targets of NO

NO has multiple targets in the brain, with the soluble form of the guanylate Cyclase (sGC) the most extensively characterized [38,48,49]. Activation of sGC subsequently increases the production of cGMP, and the level of cGMP in the cerebellum, striatum and hippocampus has been shown to depend largely on the NOS activity [50–52].

Some physiological effects of NO are, however, independent of sGC activation, and it has been demonstrated that NO, induced by NMDA receptor stimulation, activates the p21 (ras) pathway of signal transduction with a cascade involving extracellular signal-regulated kinases and phosphoinositide 3-kinase [53,54]. These pathways are known to be involved in transmission of signals to the cell nuclei and may therefore form a basis of a generation of long-lasting neuronal responses to NO. Other enzymes that constitute cellular targets for NO are cyclooxygenases, ribonucleotide reductase, some mitochondrial enzymes and NOS itself [55,56]. Finally, NO can nitrosylate proteins and damage the DNA [54,57–59].

3. NO and Psychiatric Disorders

Patients suffering from depression have been shown to have a reduced number of NOS containing neurons in the hypothalamus [60,61] and hippocampus [62]. In samples from suicide attempters, increased NO metabolites (NO_2 and NO_3) have been observed [63]. Moreover, a decreased platelet NOS activity and plasma NO metabolites in depressed patients [64,65] and a changed L-arginine

metabolism in Bipolar Disorder have been reported [66]. In addition, human genetic association studies have repeatedly found association with NO signaling and psychiatric disorders [11,67].

4. NOS inhibitors: Evidence for Efficacy in Depression and Anxiety

Over the past two decades, a number of preclinical studies have demonstrated that inhibition of NOS produces anxiolytic and antidepressant-like behavioral effects in a variety of animal paradigms. These studies include systemic injections as well as targeted infusions into the brain. The studies are primarily acute studies, and there is a great need for examination of the chronic effects. Only a few very limited clinical studies are available, which are confounded by the nonselectivity of the drug used. However, as already mentioned there are several human studies indicating an important role of elevated NO in the pathogenesis of affective disorders and anxiety, suggesting that a positive role of inhibition may be possible. Below, the results from the different NOS inhibitors used are reviewed. See also Table 1.

4.1. NOS inhibiting amino acids.

The typical NOS inhibiting amino acids associate with the substrate binding site for L-arginine [68]. The inhibitor will compete with L-arginine, and usually extra arginine will reverse the NOS inhibition produced by the inhibitor.

The best investigated compounds in this family are L-NNA (L-NG-nitroarginine), its methyl ester L-NAME), L-NMMA (L-NG-monomethylarginine) and NG-propyl-L-arginine. L-NAME requires hydrolysis of the methyl ester by cellular esterases to become a fully functional inhibitor [68]. Acute antidepressant effects have been found in both rats and mice models. L-NNA and L-NAME have thus been reported to be effective in both the Forced Swim Test (FST) and Tail Suspension Test (TST) in mice [69, 70], and in the FST in rats [71,72]. The effect of the drugs seems to display a U-shaped pharmacology, where both low and high doses have no effect [69,70,73]. Pretreatment with L-Arg has the ability to counteract the behavioral effects of the L-NAME and L-NNA [69,70,71,74], but has also been reported in some studies to have an antidepressant-like effect by itself [69].

Table 1. NOS inhibitors and studies in paradigms of depression and anxiety based on chemical class.

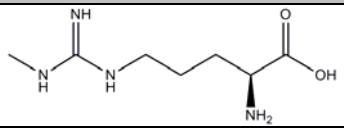
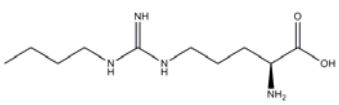
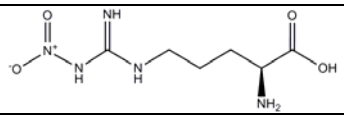
INHIBITOR AMINO ACIDS	ENZYME/ POTENCY	DRUG STRUCTURE	DEPRES-SION	ANXIETY	DRUG REF
L-NMMA or L-NANA (L-N^G-Methyl-L-arginine)	nNOS=eNOS>>iNOS		[70]	-	[68]
N-PLA (L-N^G-Propyl-L-arginine)	nNOS>>eNOS>>iNOS		-	-	[75]
L-NNA (L-N^G-Nitroarginine)	nNOS>eNOS>>>iNOS		[69, 70, 72, 76, 77, 78]	[79, 80, 81]	[68]

Table 1. *Cont.*

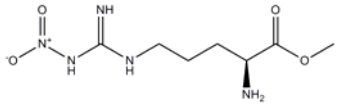
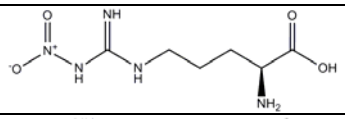
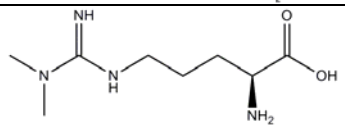
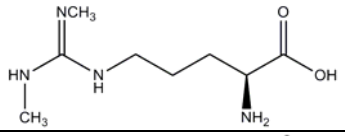
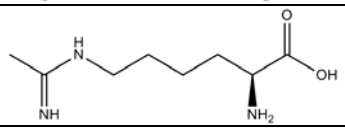
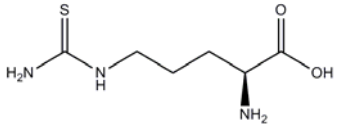
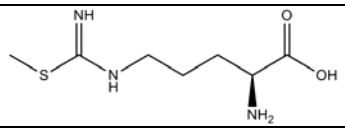
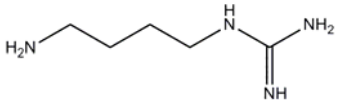
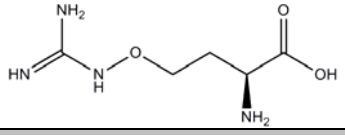
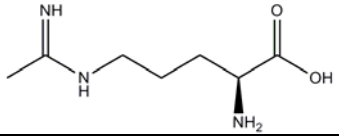
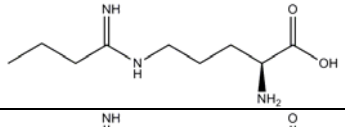
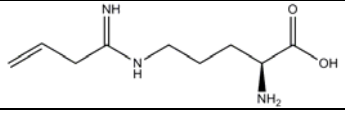
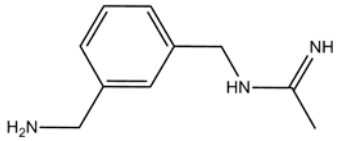
L-NAME (L-N^G-Nitroarginine methyl ester)	nNOS>eNOS>iNOS		[70, 71, 82, 83, 84, 85, 86]	[81, 87, 88, 89, 90]	[68]
L-NAA NG-Amino-L-arginine	nNOS>eNOS>iNOS		-	-	[91]
ADMA (N^G,N^G-Dimethyl-L-arginine)	-		[92]	-	[93, 94]
SDMA (NG,NG′-Dimethyl-L-arginine)	-				
L-NIL (L-N6-(1-Imino-ethyl)lysine)	iNOS>>nNOS		-	-	[95]
L-Thiocitrulline	nNOS>iNOS>eNOS		-	-	[96]
S-Methyl-L-Thiocitrulline	nNOS>eNOS>iNOS		-	-	[97]
Agmatine (1-Amino-4-guanidinobutane)	Unspecific NOS inhibitor and ligand at imidazoline receptors		[98, 99, 100, 101, 102, 103, 104, 105]	[102, 106, 107]	[108]
L-Canavanine	iNOS		-	-	[109]
AMIDINES					
L-NIO Nδ-(Iminoethyl)-L-ornithine	nNOS>eNOS=iNOS		-	-	[110]
Ethyl-L-NIO	nNOS>iNOS>eNOS		-	-	[111]
Vinyl-L-NIO	nNOS>>eNOS>iNOS		-	-	[111]
1400W (*N*-(3-(Aminomethyl)benzyl) acetamidine)	iNOS>>>nNOS>eNOS		-	-	[112]

Table 1. *Cont.*

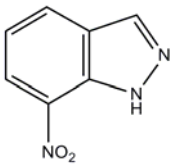
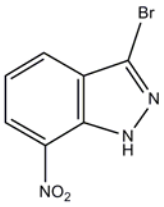
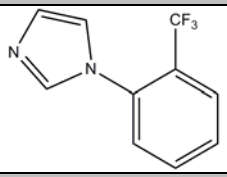
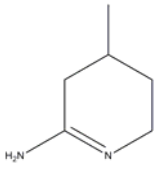
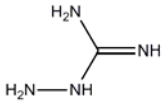
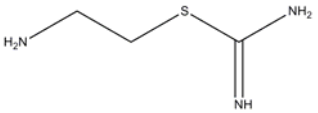
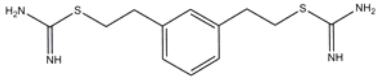
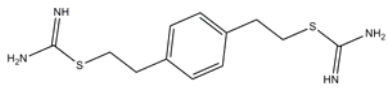
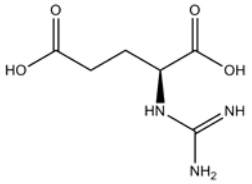
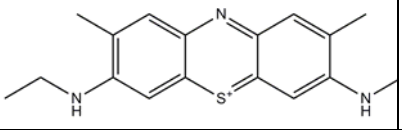
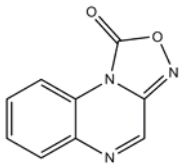
INDAZOLE DERIVATES					
7-NI (7-Nitroindazole)	nNOS=eNOS>>iNOS		[72, 89, 113, 114, 115, 116, 117, 118]	[89, 119, 120, 121, 122, 123]	[124, 125]
7-NI-Br (7-Bromonitroindazole)	nNOS>eNOS>iNOS				
IMIDAZOLE DERIVATES					
TRIM (1-[2-(Trifluoromethyl)phenyl-imidazole	iNOS=nNOS>eNOS		[115, 126, 127]	[128].	[129]
2-IMINOPIPERIDINE DERIVATES					
2-Imino-4-methylpiperidine	iNOS>nNOS>eNOS		-	-	[130]
HYDRAZINE DERIVATES					
Aminoguanidine	iNOS>>nNOS		[98, 131] [132]	[133]	[134]
ISOTHIOUREAS					
S-(2-Aminoethyl) isothiourea	iNOS=nNOS=eNOS		-	-	[135]
1,3-PBIT (S,S'-(1,3-Phenylene-bis(1,2-ethanediyl))bis-isothiourea)	iNOS>>nNOS>eNOS		-	-	[135]
1,4-PBIT (S,S'-(1,4-Phenylene-bis(1,2-ethanediyl))bis-isothiourea)	iNOS>nNOS>>eNOS		-	-	[135]

Table 1. *Cont.*

α-Guanidinoglutaric Acid	-		-	-	[136]
OTHER/MIXED					
Methylene blue	NNOS=eNOS=iNOS sGC MAO		[118, 137, 138, 139, 140]	[137, 141]	
ODQ ([1H-[1,2,4]Oxadiazole[4,3-a]quinoxalin-1-one] -	Inhibits NO sensitive cGMP formation		[116, 142].	[121]	

The clinically important features in depression, cognition and memory, have been extensively examined, and a major role for NO in the formation of memory and as a mediator in synaptic plasticity has been suggested [143,144]. A majority of studies support a facilatory role of NO in learning processes, and nNOS has been proposed to be the principal source of this retrograde messenger during long-term potentiation (LTP) [22,145], a highly important process for memory formation [146–148]. However, some controversy about this finding exist, as LTP in hippocampus and cerebellum were reported to be normal in nNOS transgenic mice [34,149]. The involvement of NOS in memory has also been confirmed in studies with NOS inhibitors. For example it was shown that systemic administration L-NAME and L-NNA impairs acquisition but not retention of spatial learning in rats [76,83,84], and L-NA reduces hippocampal mediation of place learning in the rat [77,78]. Similarly, intrahippocampal administration of L-NAME impairs working memory on a runway task without affecting reference memory [85, 86], and L-NAME has been shown to disrupt learning of an associative memory task, the conditioned eyeblink response in rabbits [83]. However, in a well-learned operant task–a delayed non-match-to-position, no effect of L-NAME was found [150], and similarly, it was also shown that L-NAME did not affect learning in a Morris Water Maze paradigm [151]. In agreement with these observations, central and systemic administration of the NO precursor, L-Arginine has been found to significantly prolong the latency time in the passive avoidance test without inhibition of locomotor and exploratory activity [152]. The interpretation of the overall neurobiological consequences of these findings remains to be established. The findings with NOS inhibitors do not initially seem correspond well with results published about other clinically relevant antidepressants, such as the SSRIs, where cognitive performance in patients have been shown to be unaffected [153] and independent from clinical recovery [154]. However, in a recent rodent study, it was reported that acute administration of imipramine and paroxetine to rats, impaired the discrimination of old from the recent objects [155]. Interestingly, following chronic administration, the imipramine-treated rats were unable to differentiate between the two objects, whereas paroxetine treated rats, as controls, spent more time exploring the old object [155]. Similarly it is, important to note that the studies with NOS inhibitors and cognitive testing predominantly have been carried out following one acute dose. The relevance for

this paradigm related to a clinical context is, as it also is the case with the other depression and anxiety tests, questionable. Only limited information is available concerning chronic administration of NOS inhibitors. However, It has been shown that L-NAME in the drinking water over 14 days impairs working memory in rats [156]. On the other hand, it has also been demonstrated that only acute, but not chronic administration of L-NAME impairs LTP formation induced by a weak near-threshold tetanus [157]. Further studies must be carried out to conclude on the overall effects of NOS inhibition on cognition.

Some of the amino-acids require special attention, as they may be considered as endogenous inhibitors. These inhibitors include L-citrulline, agmatine, NG, NG-dimethyl-L-arginine (ADMA), and argininosuccinic acid. While L-citrulline is a very weak inhibitor, a derivate, L-thiocitrulline is much more powerful [96]. Agmatine is de-carboxylated arginine [158], and has gained significant attention as there is evidence of antidepressant effects in preclinical animal models of depression [99,101–103], as well as studies suggesting a key role of agmatine in humans [98,105,159,160]. It is here, however, noteworthy to mention that agmatine also has been conceptualized as an endogenous clonidine-displacing substance of imidazoline receptors [161,162], and to have affinity for several transmembrane receptors, such as α2-adrenergic [163], imidazoline I1 and glutamatergic NMDA receptors [108]. Therefore, the effects observed in the preclinical studies may be mediated via these pathways, and not linked to NOS.

No solid preclinical data exist for the other endogenous inhibitors, although there are reports of their presence in animals [164]. Several human association studies have been published, especially regarding NG-monomethyl-L-arginine (SDMA) and ADMA [165, 166, 167]. Indeed, reports have shown an increased level of ADMA concentration in sepression, schizophrenia and Alzheimer's disease [92,168,169]; however, it is not clear whether this association is clinically important. Taken together, despite the human studies predominantly are studies carried out on peripheral tissue samples (e.g. plasma or serum), a role for the NO system in psychiatric disease is supported.

Within the field of anxiety, several interesting—but contradictory—findings have been observed, using different paradigms and drugs. For example, it has been suggested that NO has an anxiolytic-like action in the elevated plus maze (EPM) following administration of L-NNA [79,80], and also that inhibition with L-NNA caused an anxiolytic-like effect, and—in the same study—an anxiogenic-like effect with L-NAME [81]. In contrast, some other studies have reported potent anxiolytic-like effects of L-NAME in EPM [87–89]. Moreover, microinjections of L-NAME and L-NA into the periaqueductal grey were shown to produce anxiolytic-like-effects in EPM, an effect which was typically bell-shaped, and could be abolished by pre-treatment with L-arginine [90].

4.2. Indazoles and Imidazole derivates

Similar to the findings with the amino acids, antidepressant-like properties have also been demonstrated with the non-amino acid compounds. The primary benefit with the Indazoles and Imidazole derivates is a potential superiority in selectivity among the different isoforms of the NOS enzymes. This was first clear when 7-nitroindazole (7-NI) was discovered [170], as it did not have a profound effect on the blood pressure [124] as most of the amino acid inhibitors. Studies suggest that 7-NI not only interacts competitively at the substrate binding site in the NOS enzyme [170], but also

competitive regarding the co-factor tetrahydrobiopterin (BH_4) [171]. As 7-NI is also a potent inhibitor of bovine aortic endothelial eNOS in vitro, regardless of the lack of cardiovascular side-effects of this compound *in vivo* [170], other more selective isoform inhibitors have been screened. Such a compound is 1-(2-trifluoromethyphenyl)imidazole (TRIM), which is described as a potent and relatively selective inhibitor of nNOS both *in vitro* and *in vivo* [172,173]. The selectivity of this compound seems to be centered around the co-factor BH_4, and the availability of BH_4 in the tissues [129].

In the FST, 7-NI and TRIM has been found to be active [72,89,113,115,116] when administered acutely. There are no effects on locomotion following administration of the compounds. Interestingly, the effects of 7-NI have been shown to be centrally based, since intrahippocampal administration of 7-NI have been shown to cause a dose-dependent antidepressant-like effect in the FST, an effect which could be prevented following intra-hippocampal co-administration of L-arginine [114]. On the other depression related domains, 7-NI have been found to induce amnesia in a passive avoidance task in the chick [117], and impair learning and memory in different tasks such as the Morris water maze, radial maze, passive avoidance and elevated plus maze tests [123,174–177]. 7-NI have also been found to produce taste aversions, and enhance the lithium based taste aversion learning in a conditioned taste aversion paradigm, an effect that was counteracted with simultaneous administration of L-arginine [118].

Within the field of anxiety, there is more agreement on the findings with the indazoles and imidazole derivates, than with the amino acid inhibitors. It was thus shown that inhibition with 7-NI caused an anxiolytic-like effect in the EPM [89,120,122,123]. Also the selective nNOS inhibitor TRIM has been shown to possess anxiolytic-like effects in EPM [115], and has been found to modulate anxiety related behavior following the unpredictable chronic mild stress procedure in mice [128].

4.3. Hydrazine derivates and amidines

These compounds have been extensively studied in relation to cardiovascular [178–182] and endocrinological diseases [183–186]. The compounds are predominantly inhibitors of iNOS, with much less activity on the other isoforms. Aminoguanidine (AG) is a hydrazine derivate and the best characterized compound [187–189], which selectively decreases cGMP levels produced by iNOS [190]. Furthermore, AG has been observed to protect against neurodegeneration produced by chronic stress in rats [191], and to prevent the impairment of learning behavior and hippocampal long-term potentiation following transient cerebral ischemia in rats [192]. Interestingly, intracerebroventricular infusion of AG prevents the depression-like behavior following a chronic unpredictable stress paradigm [131]. Supporting these findings, a model of Post Traumatic Stress Disorder (PTSD) seems to involve exclusively the iNOS isoform, as only aminoguanidine, but not 7-NI, was effective in attenuating neurobiological readouts [132]. Together, these findings highlight the possible involvement of an inflammatory nature in depression and anxiety, which is not surprising due to the significant involvement of stress in the pathophysiology of the disorders. AG has also recently been demonstrated to display anxiolytic-like effects in EPM, open field test, light/dark test and social interaction test in stressed mice [133]. Whether these effects are present in the absence of stress remains to be established.

4.4. Other compounds/mixed

Within this group we find the only compounds proven to be effective in patients [139,140,193]. Methylene Blue (MB) oxidizes protein-bound heme and non-heme ferrous iron [194], inhibiting the stimulation of soluble guanylyl cyclase (sGC) by NO and nitrovasodilators [195]. MB was as early as 1899 described to have a calming—probably antipsychotic—effect in patients [196]. However, more recent work has focused on the beneficial effects of MB in manic-depressive disorder, where a response of 63% among 24 lithium refractory patients was found [138]. The studies were supplemented and expanded, confirming this action [139,140,193]. At the time of the study, the mechanistic hypotheses were based on changes in the vanadium ion [197–200]. Unfortunately, the studies cited above were not fully randomized, but luckily such trials are being carried out in these years [201]. It was in 1993 demonstrated that MB potently inhibited NOS both *in vitro* [202,203] and *in vivo* [204].

Several preclinical studies confirm a positive effect of MB in the FST and EPM [137], however with a U-shaped dose-response efficacy curve. Metylene blue have been demonstrated to produce taste aversions in a conditioned taste aversion paradigm, an effect comparable to the effects of 7-NI, which also could be cunteracted with simultaneous administration of L-arginine [118]. As indicated by the mode of action, MB is expected to be a very non-selective compound. Indeed, MB not only inhits NOS and sGC, but also several other heme containing enzymes, like mono-amine oxidase. In agreement with this, MB has been characterized as a potent inhibitor Monoamine Oxidase (MAO) [205–207] and various cytochromes. This effect is probably the explanation of case-reports suggesting a hyperserotonergic state following use of MB [206,208], and can be an explanation for the clinical efficacy.

Since MB also affects the NO downstream signaling pathway, including sGC, it is here worth to mention a few compounds mediating the, which affect sGC, but not NOS. Studies with selective (i.e. non-NOS) inhibitors of NO dependent cGMP formation with [1*H*-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one] (ODQ) have proven to produce antidepressant-like effects in the FST [116], as well as prevention of pro-depressant effect of L-arginine in the FST [142]. Similarly, ODQ have been shown to have anxiolytic-like properties, with an increase in the % time spent on the open arm in EPM following administration of the drug [121]. These findings are in agreement with other studies showing that an increase in cGMP, following inhibition of phosphodiesterase type V, using sildenafil, can produce anxiogenic-like responses in the EPM [209,210]. The mechanisms regarding cGMP may, however, not be easily understood, as also antidepressant actions of sildenafil have been shown following central muscarinic receptor blockade [211].

5. Interactions between NO and the Conventional Neurotransmitters

Several in vivo studies have demonstrated that NO may modulate the extracellular level of various neurotransmitters in the central nervous system, e.g. serotonin (5-HT), dopamine (DA), γ-aminobutyric acid (GABA), and glutamate [212–218]. In addition, NO can inactivate the rate limiting enzyme in the synthesis of 5-HT, tryptophan hydroxylase [219,220] and it has been suggested to stimulate synaptic vesicle release from hippocampal synaptosomes [221,222]. Furthermore, NO regulates 5-HT reuptake [223–225], inhibits uptake of [3H] DA by striatal synaptosomes [226,227] and transforms 5-HT into

an inactive form [228] . More recently, it was demonstrated that a physical interaction between the serotonin transporter and neuronal nitric oxide synthase may underlie reciprocal modulation of their activity [229]. The connection between NO and 5-HT is substantiated by observations from neurology, where studies has shown that NO as well as 5-HT is involved in the pathophysiology of migraine [230–233].

Interestingly, it has also been reported that L-Arg antagonizes the effects of the classic tricyclic antidepressant, imipramine [70]. This observation has led to hypotheses regarding the potential contribution of serotonergic/noradrenergic mechanisms in the observed antidepressant-like effects of the NOS inhibitors. Subsequently, it has been demonstrated that low and ineffective doses of L-NAME were able to potentiate the behavioral effects of imipramine and fluoxetine but not reboxetine, a norepinephrin reuptake inhibitor, in the FST [72,234]. In addition, it was shown that a serotonergic mediation of the antidepressant-like effects of L-NA, 7-NI was present, since serotonergic depletion abolished the antidepressant-like effect of the inhibitors [72]. Not all inhibitors seem to display this profile, as it also was demonstrated that the effect of agmatine was independent of 5-HT depletion [99]. However, as already discussed, agmatine may have multiple effects on several receptorsystems. Finally, NO have also been implicated in the antidepressant role of several other substances, like tramadol [235], bupropion [236], and lithium [237]. Similarly, established antidepressants, like imipramine, paroxetine, citalopram and tianeptine have all been shown to inhibit hippocampal NOS activity in vivo when applied locally in the brain [238].

6. Conclusions

Although the studies cited in the current review utilize several different compounds, affecting the different isoforms of NOS differently, the physiological role of NOS inhibition remain relatively clear. Therefore, the conclusion of the current review is that despite significant challenges in developing compounds which may differentially inhibit the ‘right’ isoform at the right place, NOS inhibition continue to be an interesting novel approach in the future development of antidepressants.

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